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Virus Activation by Vitamin A and NO_2 Gas Exposures in Hamsters

by J. C. S. Kim*

Hamsters exposed to 10 ppm NO_2 for 5 hr once a week for 4 weeks while undergoing acute vitamin A deficiency showed much reduced epithelial cell regeneration in the terminal bronchioles. Quantitative analysis done by autoradiography and scintillation counting from lung tissues indicates much reduced cell kinetics occurring in terminal bronchiolar alveolar region. Alveolar necrosis was often observed and no type II cell reversion occurred. Virus particles were found within the alveolar epithelial plasma membrane.

Lung damage due to NO_2 occurring in the distal functional unit of the rat has been studied by electron microscopy (*1, 2*). Chronic exposure to NO_2 causes epithelial cell hyperplasia which reaches a maximum level after 24 hr after post sacrifice. High level of NO_2 causes damage to alveolar macrophages, inactivates enzymes, leads to edema, and promotes infection (*3*). Furthermore, nitrogen dioxide prevents the regeneration of lost cilia (*4*). After this initial toxic insult is removed, a reparative adaptive phase follows.

That the effect of nitrogen dioxide on pulmonary tissue would be influenced by nutritional status has not been explored, although the protective effect of vitamin E as an antioxidant agent has been investigated by continuous exposure of rats fed on a defined diet (*5*). Except for the few studies with vitamin E, most respiratory inhalation experiments have been conducted with animals fed on commercial diets which not only contain essential vitamins but also protein and other fibers.

Although the modifying role of vitamin A has been extensively investigated in connection with lung cancer (*6*), no similar data are available concerning the combined toxic effect of air pollutant gases and vitamin A deficiency. Since the irritant gases first damage respiratory epithelial cells, the repair of damaged epithelial tissue may depend on adequate supply of vitamin A.

In this report we summarize our findings concerning the role vitamin A deficiency plays in cell repair followed by NO_2 exposure and observation of virus particles by combined nutritional and environmental factors.

Materials and Methods

Twenty pregnant Syrian hamsters were obtained through the courtesy of the Mammalian Genetic Division, National Institute of Health. Upon arrival, they were placed on a commercially prepared vitamin A-free diet (Tekland Diet, Madison, Wisc.) to deplete as much vitamin A stored in their liver as possible. After weaning (21–23 days post partum) the animals were placed on a vitamin A-free diet until the termination of this experiment. Weaned hamsters were sexed and grouped. One group of animals, 30 each, was maintained in vitamin A free diet and the other group was fed 50 μg of vitamin A intragastrically twice a week. For further comparison, another group of 10 animals was fed commercial Purina hamster chow. Two thirds of each group of animals were exposed to NO_2 and the others were not exposed.

NO_2 gas at a concentration of 2,040 ppm was obtained from a commercial source (Madison Gas Products, Chicago, Ill.). This gas was further diluted to 10 ppm with ambient air controlled with a flow meter prior to entering plastic rat cage measuring 14 × 12 in. The relative humidity inside the gas chamber measured approximately 50% throughout the experiment and ambient temperature ranged from 70 to 72°F. The gas flow rate was set to achieve approximately 14 air volume changes per hour. Concentration of the gas mixtures was

*Department of Pathology, Michigan State University, East Lansing, Michigan 48824.

tested colorimetrically according to the method of Saltzman (*8*) and in addition, by use of a gas detection kit (Madison Gas Products, Chicago, Ill.) at 1-hr intervals during the 5-hr exposure. Animals were chosen for gas exposure, both on a vitamin A-deficient and nondeficient diet regime, were divided into groups of 10 animals each. They were placed in the gas chamber and were exposed to 10 ppm of NO_2 for 5 hr once a week for 4 weeks.

Each group of animals was weighed weekly. Average group values were carefully followed and correlated to their health conditions. In order to monitor the vitamin A status with the onset of vitamin A deficiency, one randomly chosen animal was sacrificed weekly. A portion of each liver was assayed for vitamin A content by using the macromethod described by Neld et al. (*7*).

After 4 weeks of exposure, hamsters on either regular or vitamin A-free diets were killed at 12, 34, 48, and 72 hr post exposure. At 50 min prior to sacrifice a dose of 3 μCi of tritiated thymidine per gram of body weight was injected intraperitoneally. The animals were then sacrificed with overdose of sodium pentobarbital.

The lung was inflated by injecting 2 cc of universal fixative through the trachea according to a previously described procedure (*9*). Lung sections were oriented for light and transmission electron microscopy according to the procedure established by Evans (*10*). In order to quantitate the DNA uptake by regenerating epithelial cells, rapid autoradiography and liquid scintillation counting techniques were employed (*11*).

Results

Electron microscopic study of the lungs of hamsters on a regular commercially prepared diet and exposed to NO_2 have shown hypertrophy and focal hyperplasia in the epithelium of the terminal bronchioles, and loss of cilia. Lung tissue obtained from two gas-exposed hamsters on a vitamin A-deficient diet for 8 weeks showed definite morphological differences. Alveolar necrosis was often observed (Fig. 1). Numerous small lipid droplets were found within the alveolar walls. The thickening of epithelial basement membranes due to edema was striking. Electron-dense bodies and calcium deposits were observed along the inner and outer surfaces of the basement membranes. The epithelial call membranes were irregularly formed, often vesiculated and protruded into the airway. "Virus particles" some of which contained a dense nucleoid core structure, measuring about 100 nm in diameter, were observed within the epithelial plasma membranes (Fig. 2). A few particles were protruding into the airway. No such particles were found in nondeficient animals exposed to the gas. These structures resemble the herpes group of viruses. A latent and indigenous herpes-like virus particle was identified previously from hamsters with proliferative ileitus syndrome (*12*).

The amount of thymidine incorporated into the lung tissue indicating DNA uptake was quantitated by both scintillation counting and autoradiography. As shown in Table 1, counts made on equal amounts (1 g) of homogenized lung tissue, indicate much reduced DNA uptake in the vitamin A-deficient group of animals. The results of autoradiographic studies indicate reduced labeling index and delayed reparative responses (Fig. 3).

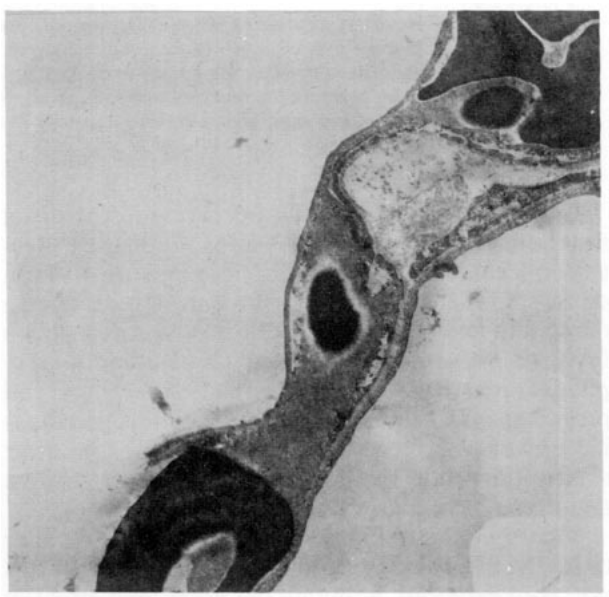

FIGURE 1. Pyknotic nuclei of the alveolar epithelium were often seen in animals deficient in vitamin A. Uranyl acetate and lead stain ×8,700.

Cells of a similar population in animals on normal diet were heavily labeled 12 hr after exposure (*13*). Histologic examination revealed a much reduced basal cell growth; moreover, basal cell growth was less extensive in the acutely deficient groups of animals in comparison to the control. In addition, the recovery index did not match that of the control group. Type II cell reversion from type I cell damage did not take place, since no lactate dehydrogenase isoenzymes III were observed in the terminal airways by special stain. Instead, numerous macrophages were found in the lumens of alveoli.

Acute vitamin A deficiency was confirmed by both liver vitamin A assay and body weight reduction. The weight loss was noted from 7 weeks on. This was correlated with a vitamin A liver content

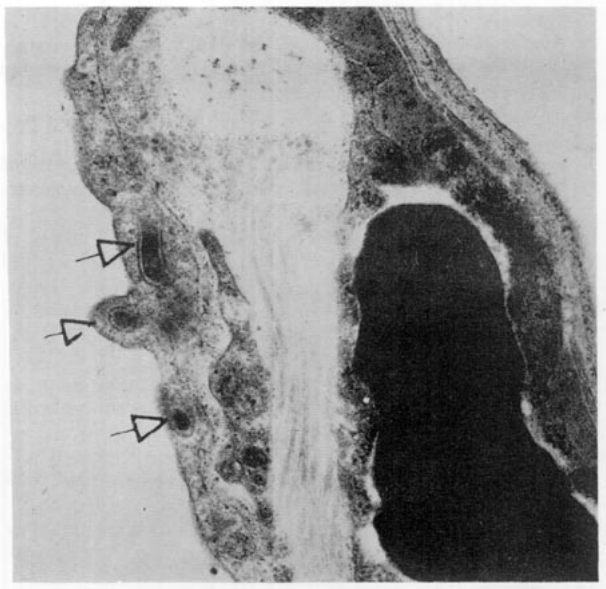

FIGURE 2. Virus particles budding from the plasma membrane with a dense nucloid structure (arrows). Uranyl acetate and lead stain ×25,000.

reduction from 32.0 to 0.9 μg/g liver weight at the time of sacrifice.

Table 1. Distribution of ^{3}H-thymidine radioactivity in lung tissue from hamsters fed regular diet and vitamin A-deficient diet monitored by means of liquid scintillation counting.

No. of animals	Diet	^{3}H-thymidine activity, cpm
17	Vitamin A-free	157
8	Regular control	546

[a]Background count was illuminized. Assay of the regular commercial Purina hamster chow homogenates for vitamin A showed 12.3 μg/g of food.

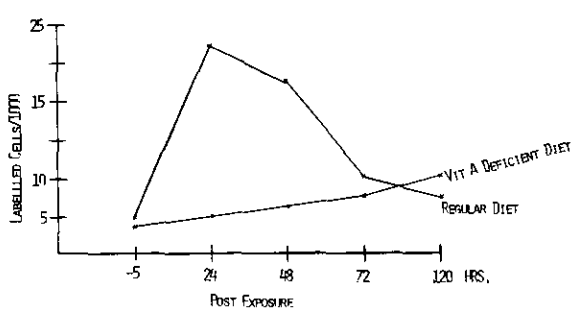

FIGURE 3. Cell cycle kinetics in bronchoalveolar region after intermittent NO_2 gas exposure in animals fed regular and vitamin A-deficient diets.

Discussion

Nutrition may play an important role in the overall health effects of pollutant gases. Through a series of laboratory experiments, we have unequivocally demonstrated the important role vitamin A plays in repair resulting from damage caused by exposure to nitrogen dioxide gas. The cell kinetics effect occurring in the respiratory tract of hamsters maintained on various levels of vitamin A was measured by autoradiography, by scintillation counting, and histopathologic techniques. More proliferative epithelial response to 10 ppm of NO_2 was seen in animals maintained on prolonged high dose levels of vitamin A, whereas NO_2 lesions of much lesser degree were observed in animals maintained on a vitamin A-free diet (*13*). In acute deficiency state, epithelial cell type reversion did not occur.

It is now established that in damage and death of the cells lining the alveolar surface, type I alveolar cells may be quickly followed by a proliferation of type II alveolar cells. This has been observed universally with chemical insults. Type II cells are apparently the critical element in repairing lung damage (*3*). It appears that they might strengthen the biochemical defense mechanisms of the pulmonary parenchyma. We should be able to determine whether vitamin A is necessary for this cell reversion. Our current undertaking strongly indicates that vitamin A is a critical ingredient for this cell reversion to take place.

Nutritional factors such as vitamin A may play an important role in activating viral genome. This aspect in comparison to other genetic and immunologic factors has not been investigated. The present result clearly implicates nutrition combined with NO_2 as one other possible factor for activating oncogenic viruses, although impaired humoral and cellular immunity due to vitamin A deficiency may contribute to such activation.

Contrary to common belief, both a selective vitamin A deficiency and chronic hypervitaminosis A created by self-medication are widespread in this country. Selective deactivation of the hepatic vitamin A storage depot within the hepatic cytoplasm by chronic ingestion of alcohol has been firmly established in man as well as in monkeys (*14*). Other hepatotoxic agents in the environment can disturb liver metabolism and damage the liver cells. Experimental findings suggest the need critically to reanalyze the essential vitamin A and other nutritional requirements for urban populations. Such an analysis will require much more experimentation in animals prior to applying such knowledge for the benefit of man.

Further research on combined effects of both environmental and host factors are needed in view of the recent epidemiological findings linking a high incidence of urban cancers to high levels of nitrogen dioxide gas in the air (*15*). Both NO and NO_2 can form nitrous acids which then combine with amine to form nitrosamines. The nitrosamines are the major suspect in urban cancer in man (*15*). Our investigations clearly indicate that vitamin A-deficient hamsters were more susceptible to NO_2 gas exposure. At the same time, the deficient animals, when exposed intermittently, showed marked lymphopenia which may cause activation of hidden virus in the lung. Nonexposed hamsters did not show these changes. Implication of this laboratory finding to human situation exists, since dietary vitamin A and human cancer has been proven by a five year epidemiologic follow-up study of 8278 Norwegian men (*16*): a positive relationship between cigarette smoking and lung cancer appeared at both high and low levels of vitamin A intake. The findings suggest that vitamin A active compounds or some closely associated dietary factors may modify the expression of pulmonary carcinogens or cocarcinogens in man in combination with air pollutant (*16*).

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